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APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO.
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08/599,226 02/09/96 SALFELD

EXAMINER
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HM11/0417

LAHIVE & COCKFIELD, LLP  
28 STATE STREET  
BOSTON MA 02109

ART UNIT	PAPER NUMBER
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EIGENSCHEMKE  
13

DATE MAILED:

04/17/98

This is a communication from the examiner in charge of your application.  
COMMISSIONER OF PATENTS AND TRADEMARKS

OFFICE ACTION SUMMARY

☒ Responsive to communication(s) filed on 2/9/98

☒ This action is FINAL.

☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 D.C. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire 3 month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

☒ Claim(s) 1-23, 44, 65 is/are pending in the application.

Of the above, claim(s) \_\_\_\_\_ is/are withdrawn from consideration.

☐ Claim(s) \_\_\_\_\_ is/are allowed.

☒ Claim(s) 1-23, 44, 65 is/are rejected.

☐ Claim(s) \_\_\_\_\_ is/are objected to.

☐ Claim(s) \_\_\_\_\_ are subject to restriction or election requirement.

Application Papers

☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on \_\_\_\_\_ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on \_\_\_\_\_ is ☐ approved ☐ disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some\* ☐ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) \_\_\_\_\_.

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

\*Certified copies not received: \_\_\_\_\_

☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☐ Notice of Reference Cited, PTO-892

☐ Information Disclosure Statement(s), PTO-1449, Paper No(s). \_\_\_\_\_

☐ Interview Summary, PTO-413

☐ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

—SEE OFFICE ACTION ON THE FOLLOWING PAGES—

1. The location of this application has changed. All future correspondence regarding this application should be sent to the Examiner's attention with the designation Technology Center 1600, Group 1640, Art Unit 1644. Current fax and telephone contact numbers may be found at the end of this Office Action.
2. This application has been filed with informal drawings which are acceptable for examination purposes only. Formal drawings will be required when the application is allowed.

3. The following is a quotation of the first paragraph of 35 U.S.C. § 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

4. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

5. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 C.F.R. 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103(a).
6. Claims 1-23 and 44 are rejected under 35 U.S.C. 103(a) as being unpatentable over Griffiths et al. (EMBO J.) In view of Lewis et al. (WO 95/23813) or Lewis et al. (J. Cell. Biochem.). Claims 1-23 and 44-46 are drawn to human TNF- $\alpha$  antibodies. Griffiths teach human antibodies to TNF- $\alpha$  derived from phage display libraries. The reference differs from the claimed invention in that the binding affinities and kinetics of

antigen binding differ from those of the claimed human monoclonal antibodies. Both Lewis references teach the use of alanine scanning mutagenesis to increase the binding affinities and improve the kinetics of antigen binding of monoclonal antibodies. Observed improvements in antigen binding as high as eleven fold have been observed by Lewis et al. (See page 3, PCT '492, about line 25). Lewis et al. also noted ten-fold slower antigen dissociation rates with antibodies produced using alanine scanning mutagenesis (see PCT '492, page 4, lines 14-22).

One of ordinary skill in the art at the time the invention was made would have been motivated to utilize alanine scanning mutagenesis to produce and select human TNF- $\alpha$  specific monoclonal antibodies having high binding affinities, slow dissociation constants, and improved antigen kinetics because such antibodies would have been useful for the production of diagnostic and therapeutic antibodies. From the teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole is prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

*Applicants' traversal of the rejection is noted and found unpersuasive for the following reasons. Applicant argues that the claimed invention possesses unexpected properties over the prior art antibodies. This argument ignores the secondary references which indicate that increases in antibody affinity and lowering of off rates of antibodies are accomplished by applying the methods of the secondary references (Lewis et al.) to antibodies of the prior art. The secondary references indicate that antibody affinity can be increased by following the teachings of the references. Applicant argues that the claimed invention possesses unexpected results as compared to the prior art or combination thereof, however it is unclear that the claimed invention is commensurate in scope with those antibodies possessing the unexpected properties. It is well settled that whether the unexpected results are the result of unexpectedly improved results or a property not taught by the prior art, the "objective evidence of nonobviousness must be commensurate in scope with the claims which the evidence is offered to support." In other words, the showing of unexpected results must be reviewed to see if the results occur over the entire claimed invention. In re Clemens, 622 F.2d 1029, 206 USPQ 289, 296 (CCPA 1980). See also MPEP §716.02(d). Applicants' claims appear to remain broader in scope than the arguments presented in traversal of the rejection. The nonobviousness of a broader claimed invention can be supported by evidence based on unexpected results from testing a narrower range if one of ordinary skill in the art would be able to determine a trend in the exemplified data which would allow the artisan to reasonably extend the probative value thereof. In re Kollman, 201 USPQ 193 (CCPA 1979). There is no evidence supporting such a conclusion in the present application.*

*Applicant also argues that the references fails to provide motivation and suggestion to make the amino acid substitutions necessary to arrive at the sequences claimed in claims 9 and 12, relying upon In re Deuel. This argument is not persuasive for the following reasons. In Deuel and Bell, the arguments were directed to exact DNA sequences for proteins having unknown numbers of encoding genes within the entire genome of an individual. Another issue within the cases was the tissue source of the DNA. The Fed. Cir. also stated in Deuel at page 1215,*

*A different result might pertain, however, if there were prior art, e.g., a protein of sufficiently small size and simplicity, so that lacking redundancy, each possible DNA would be obvious over the protein. See In re Petering, 301 F.2d 676 (CCPA 1962) (prior art reference disclosing limited genus of 20 compounds rendered every species within the genus unpatentable).*

*These facts differ significantly from the facts of this application in that there is only one single functional gene encoding the Ig molecule within the hybridoma, said hybridoma available to the routineer before the filing date of this invention. Further genes encoding Ig molecules have been isolated from hybridomas for a number of years. The secondary references teach the substitution of alanine residues for the increase of antibody binding affinity. Applicant has taken a known antibody molecule and subjected the DNA encoding this molecule to known in vitro methods/techniques to increase the binding affinity of the antibody molecule. Further, Griffiths teaches the DNA sequences of TNF specific antibodies (Table 2). The DNA molecule which would have been obtained by following the combination of references would have identified those amino acid residues which should be mutated to alanine residues for increasing the binding affinity of the antibody molecule. Based on the combination of references, one of ordinary skill in the art would have had a reasonable expectation of success in arriving at the invention as currently claimed. A person having ordinary skill in the art, versed in the field of molecular biology and the use of recombinant DNA techniques is presumed to be familiar with technology and techniques in the field of cloning at the time the invention was made, including (1) rapid advances in the field of cloning discussed in Amgen, Inc. v. Chugai Pharmaceutical Co., 13 USPQ2d 1737, 1753-54 (D. Mass. 1989), and (2) more recent techniques of DNA cloning discussed in the references cited above. The references provide suggestion and motivation to make humanized antibodies and the combination of references provide a reasonable expectation of success in arriving at the claimed invention. Further, the Deuel and Bell cases were decided in view of the state of the art at the time those inventions were filed in 1990 and 1987, respectively. The state of the art has advanced considerably since that time. Applicant is invited to consider Ex parte Goldgaber, 41 USPQ2d 1172 (Bd. Pat. App. Int. 1996). In Goldgaber, the genomic starting material was commercially available and the references place the routineer in possession of the probes necessary*

*for the isolation of the cloned gene. The combination of references in this rejection, combined with the public availability of antibody starting materials (see specification, page 22, around line 25-30) places the routineer in possession of the genetic starting material necessary for the production of the claimed recombinant DNA molecules and all the required teachings necessary to arrive at the DNA encoding the recombinant antibodies. The combination of references provides all that is necessary for a finding of obviousness, motivation to combine the references and a reasonable expectation of success. Thus, for the foregoing reasons, Applicants' arguments have been considered and found unpersuasive.*

7. Claims 1-23, 44, and 65 are rejected under 35 U.S.C. 103(a) as being unpatentable over Griffiths et al. (EMBO J.) in view of Lewis et al. (WO 95/23813) or Lewis et al. (J. Cell. Biochem.), as applied above and further in view of Adair et al. (WO 92/11383). Claims 44 and 65 are directed to pharmaceutical compositions comprising the antibodies of claims 1-23 along with another active ingredient. The teachings of the Griffiths, and Lewis references have been discussed supra. Adair teaches compositions of humanized anti-TNF antibodies along with other active ingredients, such as xanthines and/or antibodies of other specificities (see page 22, paragraph 3). The additional components of the composition of Adair would have been recognized by one of ordinary skill in the art as components suitable for the amelioration of septic shock, reduction of immune system cells, and/or the inflammatory response. Thus, one of ordinary skill in the art would have been motivated to substitute the antibodies which would have been obtained through the combination of the Griffiths and Lewis references for the anti-TNF antibodies of Adair to arrive at compositions similarly useful for the treatment of disease according to the teachings of Adair. From the teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole is prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.
8. No claim is allowed. Applicant's amendment necessitated the new grounds of rejection. Accordingly, **THIS ACTION IS MADE FINAL**. See M.P.E.P. § 706.07(a).

A SHORTENED STATUTORY PERIOD FOR RESPONSE TO THIS FINAL ACTION IS SET TO EXPIRE THREE MONTHS FROM THE DATE OF THIS ACTION. IN THE EVENT A FIRST RESPONSE IS FILED WITHIN TWO MONTHS OF THE MAILING DATE OF THIS FINAL ACTION AND THE ADVISORY ACTION IS NOT MAILED UNTIL AFTER THE END OF THE THREE-MONTH SHORTENED STATUTORY PERIOD, THEN THE SHORTENED STATUTORY PERIOD WILL EXPIRE ON THE DATE THE ADVISORY ACTION

IS MAILED, AND ANY EXTENSION FEE PURSUANT TO 37 C.F.R. § 1.136(a) WILL BE CALCULATED FROM THE MAILING DATE OF THE ADVISORY ACTION. IN NO EVENT WILL THE STATUTORY PERIOD FOR RESPONSE EXPIRE LATER THAN SIX MONTHS FROM THE DATE OF THIS FINAL ACTION.

9. Papers related to this application may be submitted to Group 1600 by facsimile transmission. Papers should be faxed to Group 1600 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). THE CM1 FAX CENTER TELEPHONE NUMBER IS (703) 308-4242.
10. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Christopher Eisenschenk whose telephone number is (703) 308-0452. The examiner can normally be reached Monday through Thursday from 6:30 am to 5:00 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christina Chan can be reached on (703) 308-3973. Any inquiry of a general nature or relating to the status of this application should be directed to the Technology Center receptionist whose telephone number is (703) 308-0196.



April 16, 1998  
Christopher Eisenschenk, Ph.D.  
Primary Examiner  
Group 1640

# Antibody Engineering: Research and Application of Genes Encoding Immunoglobulins

**T 522 LIPID-TAGGED ANTIBODIES FOR IMMOBILIZATION ONTO LIPID BILAYERS**, Marja-Leena Laukkanen and Karl Keinänen, VTT Biotechnical Laboratory, P.O.Box 202, SF-02181 Espoo, Finland

We have studied possibilities to anchor antibody fragments onto lipid bilayers via hydrophobic tags for immunodiagnostic and other applications. Previously we reported that a bacterially produced anti-2-phenytoxazone single-chain Fv antibody fragment (Ox scFv, Takkinen *et al.*, 1991) can be converted by genetic engineering into a membrane-anchored protein by fusion with the major lipoprotein (lpp) of *E. coli* (Laukkanen *et al.*, 1993). The resulting antibody fragment (Ox lpp-scFv) contains an N-terminal covalently bound lipid tag and is stably associated with bacterial cell envelope and displays antigen-binding activity in membrane-bound as well as detergent-solubilized form.

In order to purify the fusion protein by immobilized metal affinity chromatography (IMAC), a lipid-tagged antibody with poly-His tail (Ox lpp-scFv-H6) was constructed. The expression of the Ox lpp-scFv-H6 in *E. coli* resulted in the production of a 30 kDa fatty acid modified protein with antigen-binding profile similar to parental Ox lpp-scFv. Purification by IMAC followed by hapten affinity chromatography yielded a highly purified lipid-tagged antibody which was reconstituted into liposomes by detergent dialysis. Liposomes carrying the antibody show specific antigen-binding activity measured as by ELISA and by real-time biospecific interaction analysis using BIAcore.

Lipid-tagged antibodies may find use in immunoliposome, vaccine and biosensor technology.

Takkinen, K., Laukkanen, M.-L., Stemann, D., Althaus, K., Immonen, T., Vainio, L., Kaartinen, M., Knowles, J.K.C. and Teeri, T.T. (1991) *Protein Engng* 4: 837-841.

Laukkanen, M.-L., Teeri, T.T. and Keinänen, K. (1993) *Protein Engng* 6: 449-454.

**T 524 CONSTRUCTION OF SINGLE-CHAIN ANTIBODY FRAGMENTS AND EVALUATION IN WHOLE-BLOOD DIAGNOSTIC KITS**, Glenn G. Lilley, Greg Cola, Olan Dolezal, Carmel Hillyard<sup>1</sup>, Dennis Rylatt<sup>1</sup> and Peter Hudson, CSIRO Division of Biomolecular Engineering, 343 Royal Parade, Parkville, Australia, 3052, and <sup>1</sup>AGEN Biomedical Limited, P.O. Box 391, Acacia Ridge, Australia, 4110.

We have designed, constructed and expressed scFv molecules that can replace Fab fragments as red blood cell agglutination reagents. The new technology has enabled the construction of antibody-like molecules with multiple functionality or altered or even increased specificity.

The SimpliRED diagnostic kit for HIV-1 (AGEN Biomedical Ltd., Australia) incorporates as the active reagent, a bifunctional, antibody-based molecule which is constructed by protein chemical techniques from the Fab portion of a mouse monoclonal antibody and a synthetic peptide epitope. The Fab domain recognises the glycoprotein, glycophorin-A found on the surface of human erythrocytes and the linked peptide epitope is in turn recognised by antibodies in the sera of individuals who have contacted and raised an immune response to HIV. The addition of the reagent to the blood of these patients causes rapid red cell agglutination.

The monoclonal antibody which forms the basis of the SimpliRED kit reagent has been cloned and the functional variable domains have been sub-cloned into *E. coli* expression vectors in the form of an scFv molecule fused to the FLAG octapeptide epitope or alternatively to HIV-1 epitopes (gp41, gp120 and p24 fragments). The products expressed in *E. coli* are recognised in Western blots by monoclonal antibodies directed against the C-terminal epitopes. The recombinant fusion protein of scFv and FLAG epitope can mimic the commercial reagent in agglutination assays. Research is directed towards the improvement of the expression system and purification methods for the use of the recombinant scFv reagents in robust diagnostic kits.

**T 523 USE OF ALANINE SCANNING MUTAGENESIS TO IMPROVE THE AFFINITY OF AN ANTI gp120 (HIV) ANTIBODY**

Craig M. Lewis, Jwu-Sheng Tung, George E. Mark, Greg F. Hollis and Steven W. Ludmerer, Department of Cellular and Molecular Biology, Merck Research Laboratories, Rahway, NJ 07065

Antibodies that recognize V3 loop peptides of HIV gp120 have been shown to neutralize HIV *in vitro* and *in vivo*. It has been demonstrated that the antibody-antigen off-rate is an important parameter in neutralization, therefore the ability to decrease the off-rate of an antibody through mutagenesis is likely to increase its therapeutic potential. We isolated several mutants of a single chain Fv antibody (clone P5Q) with reduced off-rates to V3 loop peptides. Critical residues of the VH CDR3 region were identified by alanine substitution mutagenesis (alanine scanning). Four classes of affinities, as determined by BIAcore analysis, were observed upon replacement of alanine at each of the 27 amino acid positions of CDR3: i) increased binding (2), ii) decreased binding (5), iii) no binding (6), and iv) no change (14) relative to P5Q. Positions that resulted in increased or reduced binding (classes i and ii), operationally defined as critical, were candidates for further study.

The two class i positions (improved off-rates) were subsequently randomized to all amino acids and optimal solutions determined. In one case the optimized improvement is observed with glutamic acid (2.8 fold improvement relative to P5Q). Smaller improvements are observed with other polar or negatively charged amino acids, while binding was eliminated with hydrophobic residue substitutions. In the second case optimized improvement was observed with tryptophan (4.7 fold), and measurable improvements were observed with several hydrophobic residues. Thus the method is not limited to just one kind of amino acid substitution. Additional studies on one of these positions demonstrates that the improvement is observed with several gp120 V3 loop peptide variants.

To date, one member of class ii (decreased binding) has been randomized. In contrast to the class i residues described above, the aspartic acid which appears at this position in P5Q is the optimal residue. To determine whether or not improvements are additive, scFv derivatives which contain optimal amino acids at some or all of these positions are being evaluated.

**T 525 IDIOTYPE MAPPING OF ANTI-DNA Fab FRAGMENTS EXPRESSED IN E. COLI**

Offen Daniel<sup>\*</sup>, Irit Kline<sup>\*</sup> and Betty Diamond<sup>\*</sup>  
Felsenstein Medical Center Bellinson Campus Petach-Tikva  
49100 ISRAEL<sup>\*</sup>, Albert Einstein College of  
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Patients with lupus erythematosus (SLE) characteristically produce antibodies to (ds)DNA. The pathogenic importance of these antibodies is suggested by their fluctuation with disease activity. Idiotype analysis of anti-DNA antibodies have been informative regarding the structural basis for DNA binding and for pathogenicity. Serum titer of 3I and F4 idiotypes, identified by monoclonal antibodies, correlate with serum levels of anti-dsDNA activity in SLE patients. This study has focused on the 2A4 antibody which possesses high affinity anti-dsDNA activity and expresses both the 3I and F4 idiotypes, located on the light and heavy chains respectively. Using PCR we have amplified the 2A4 CH1, VH4 and Cκ, VK1 genes, 700bp each, and insert them separately or together into the pCOMB-3 plasmid vector. Several *E. coli* clones expressing light chain, heavy chain and Fab fragments were obtained. High level expression can be seen both in bacteria cells and supernatants. Deletions and directed mutagenesis are being performed now in order to identify the exact idiotypes sequences. This antibody engineering approach provides a means for idiotype analysis and may identify the correlates to idiotype and antigenic specificity and pathogenicity.